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Testing the effectiveness of microbial biostimulants to increase the resistance of the variety Chardonnay to "Bois noir" phytoplasma

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Abstract

Bois noir (BN) of the grapevine is one of the phytoplasma diseases that have become increasingly prevalent in Czech vineyards in recent years, and causes economically significant damage. As no curative measures are currently known, indirect control methods are being sought to help reduce the negative impact on the vine and extend the life of the vineyard. In a trial with the variety Chardonnay, the following biostimulants were tested: NovaFerm® SIRIUS (5.0 l/ha), NovaFerm® ORION (5.0 l/ha) and Microfertile Carbon (5.0 l/ha), in the periods 2023 and 2024. When comparing plants with BN symptoms, the lowest number of plants with BN symptoms was found in the NovaFerm Orion variant. The NovaFerm Orion belongs to microbial biostimulants and shows a significant biostimulatory effect on growth, as there was a decrease in weakly growing plants during the growing season. When evaluating the effect on plant recovery, the biostimulant Microfertile Carbon showed the most significant effect, leading to a year-on-year recovery in the number of symptomatic plants.

Keywords: grapevine, Bois noir, biostimulants, recovery

Zusammenfassung

Untersuchung der Wirksamkeit mikrobieller Biostimulanzien zur Erhöhung der Resistenz der Rebsorte Chardonnay gegen das Phytoplasma „Bois noir". Bois Noir (BN) bei Weinreben ist eine Phytoplasmose, die in den letzten Jahren in den Weinbergen der Tschechischen Republik wieder verstärk auftritt und erhebliche wirtschaftliche Schäden verursacht. Da es keinen direkten Schutz gegen diese Krankheit gibt, werden indirekte Schutzmaßnahmen erforscht, um die negativen Auswirkungen auf die Reben zu minimieren und die Lebensdauer der Weinberge zu verlängern. In den Jahren 2023 und 2024 wurden in einem Versuch mit der Sorte Chardonnay die folgenden Biostimulanzien getestet: NovaFerm® SIRIUS (5,0 l/ha), NovaFerm® ORION (5,0 l/ha) und Microfertile Carbon (5,0 l/ha). Die geringste Anzahl an Pflanzen mit BN-Symptomen war bei NovaFerm Orion zu finden. Das Produkt NovaFerm Orion gehört zu den mikrobiellen Biostimulanzien und zeigt eine deutliche biostimulierende Wirkung auf das Wachstum, da ein Rückgang schwach wachsender Pflanzen beobachtet wurde. Bei der Bewertung der Pflanzenerholung zeigte Microfertile Carbon die stärkste Wirkung. Es führte zu einer Reduktion der Anzahl der symptomatischen Pflanzen von Jahr zu Jahr.

Schlagwörter: Rebe, Bois noir, Biostimulanzien, Erholung

Introduction

Bois noir (BN) of the grapevine is an important phytoplasma-associated with grapevine yellows (GY) that causes significant economic losses regarding yield and quality of grapes, in most wine growing regions (Maixner, 2011). Bois noir has been associated with 'Candidatus Phytoplasma solani' (CaPsol, subgroup 16SrXII-A or stolbur group) (Quaglino et al., 2013, Angelini et al., 2018).

The stolbur phytoplasma is able to infect a wide range of more than 90 species of cultivated and weedy plants. The most important host plants in Europe are the stinging nettle (*Urtica dioica* L.) and the field bindweed (*Convolvulus arvensis* L.). They are also the primary hosts of the stolbur phytoplasma and its main vector, cixiid planthopper (*Hyalesthes obsoletus*). These reservoir species of perennial plants have great importance in epidemiology as reservoir species for the pathogen (Dermastia et al., 2017).

The symptomatology of stolbur phytoplasma is almost identical to other grapevine yellows (Belli et al., 2010). The main symptoms are visible on leaves, shoots, flowers and cluster. The first easily confusable symptoms appear after bud burst. In heavily infected plants, there is necrosis of terminal buds, shortening of internodes, fading of leaves and poor lignification of shoots with occasional black blisters. The flowers very often wither and fall off (Kölber, 2011).

Disease incidence is also temporally variable with alternating epidemic and endemic years. The main factor of variability in disease incidence is the strongly varying infection pressure depending on the host plant (Maixner, Kröhner & Kappel, 2011).

The planthopper, *Hyalesthes obsoletus* Signoret, 1865 (Hemiptera: Cixiidae) is a major vector and driver of *'Ca. Phytoplasma solani*' epidemiology (Kosovac et al., 2019). This insect is a polyphagous species that, as an adult, feeds on many plant species. Reproduction of this insect occurs exclusively on weedy vegetation.

The presence of *Hyalesthes obsoletus* is not restricted to the vineyard but depends on the presence and distribution of host plants inside and outside the vineyard. Infection pressure is not only determined by the conditions of an individual vineyard, but also depends on the general biotic and abiotic conditions over a large area (Bianco et al., 2019). Transmission of stolbur from vine to vine has not been observed, so that the grapevine is the dead end host of stolbur (Kölber, 2011). Therefore, the occurrence of stolbur in a vineyard does not affect the further spread of the disease and there is no need to remove infected vines from the vineyard (Pavan et al., 2012). Infection pressure in a vineyard is rather determined by the presence and infestation of host plants and the presence of the respective vector (Maixner and Mori, 2013).

The complexity of the epidemiology of BN makes it difficult to design effective control strategies. Strategies used directly in the vineyard to reduce the spread or incidence of BN are based on (i) preventive removal of annual shoots on which *H. obsoletus* might feed after mulching of the cover crops; (ii) rejuvenation of trunk in the vineyard; (iii) treatment with resistance-inducing agents (Mori et al., 2015).

In general, management of phytoplasma diseases is challenging and, in most cases conventional control strategies are not directly directed against the pathogen. Depending on the given situation, they focus on eradication of the infected reservoir plants and insecticide treatments against the vectors (Riedle-Bauer and Brader, 2023).

An important method for enhancing plant defence responses against symptom-reducing pathogens is the application of resistance inducers, also known as elicitors. Resistance inducers can activate stress-related defence pathways in plants and modify plant-pathogen-vector interactions affecting the emission of plant volatile compounds capable of attracting or repelling insect vectors (Dicke and Hilker, 2003; Minuz et al., 2020). Therefore, some studies have been carried out using elicitors to trigger defence responses in grapevine in relation to phytoplasmas and recovery within the plant (Oliveira et al., 2019). Also, fungal and bacterial endophytes have been tested to affect grapevine recovery from grapevine-yellows (Martini et al., 2009; Bulgari et al., 2016).

Biostimulants activate physiological processes in plants, improve nutrient acceptability, stimulate plant development and reduce the use of fertilizers (Kunicki et al., 2010). The tested biostimulants were based on chitosan (Romanazzi et al., 2009, Romanazzi et al., 2013), benzithiadiazole and a mixture of glutathione and oligosaccharides (Romannazzi et al, 2013), fosetyl-Al and a mixture of glutathione and oligosaccharides (Garau et al., 2008) and the application of humic and fulvic acids and algae extract (Mazio et al., 2008). Biostimulants based on beneficial fungi or growth promoting bacteria have an influence on plant metabolism, leading to improved plant growth, resistance to stress conditions, improved nutrient utilization and production quality (Teklic et. al., 2020). Microbial biostimulants do not accumulate long-term residues, have low phytotoxicity, and are less susceptible to selection by resistant strains of pests and diseases, and therefore can have significant environmental and human benefits (Sangiorgio et al., 2020).

An interesting phenomenon that can occur in grapevine infected with phytoplasma is "recovery", the spontaneous decline of disease symptoms in plants that previously exhibited them (Caudwell, 1961). In grapevine, this natural phenomenon has been observed in several varieties and wine regions at different levels (Romanazzi et al., 2013).

Riedle-Bauer et al. (2010) presumed that the visual symptoms reflected the internal pathogen titre and the distribution of the phytoplasmas in the vine. They observed a direct correlation between degree of BN symptoms and recovery rates.

One way to improve control of Bois noir is to understand the very complex epidemiological cycles that involve multiple '*Ca. Phytoplasma solani*' host plants and insect vectors, the most important of which is *H. obsoletus* (Mehle at al., 2022).

The aim of this experiment was to evaluate the effect of the use of biostimulants on the development of BN in the vineyard with the sensitive variety Chardonnay. The development of BN in the vineyard and the influence of the vine recovery process were also mapped.

Materials and methods

The experimental vineyard is located in the Sonberk winery, vineyard site: Sonberk, wine-growing village: Popice, wine-growing sub-region Mikulov. The vineyard are situated on loessal soil at an altitude of about 230 m above sea level with an orientation to the south and south-west. The vineyard is cultivated according to the rules for integrated production (IP) valid in the Czech Republic. The experiment was conducted with the variety Chardonnay, planted in 2012, the rootstock is SO4. The spacing is 2,2 x 0,9 m with trellis system: vertical shoot position.

The following biostimulants were evaluated (Tab. 1): NovaFerm® SIRIUS (5.0 l/ha), NovaFerm® ORION (5.0 l/ha) and Microfertile (5.0 l/ha).

Tab. 1: Characteristics of used biostimulants.

Name	**Producer**	**Characteristic**
NovaFerm® SIRIUS	Nova Scienta Kft., H-6230, Soltvadkert Kiss Ernő út 75 Hungary	Microbial strain: *Bacillus licheniformis* (min. 1×10^7 cfu/ml) - max. 5%. pH value: 6.5 – 7.5 UV-resistant, good tolerance to low and high temperatures, no risk of resistance, zero safety period.
NovaFerm® ORION	Nova Scienta Kft., H-6230, Soltvadkert Kiss Ernő út 75 Hungary	Microbial strain: *Bacillus thuringiensis* spp. *kurstaki*, *Bacillus thuringiensis spp tenebrionis* (1×10^9 cfu/ml), *Photorhabdus luminescens* (1×10^9 cfu/ml) pH value: 6.5 – 7.5 UV-resistant, good tolerance to low and high temperatures, no risk of resistance, zero safety period.
Microfertile Carbon	Ekolive, Paderborn, Germany	Microbial strain: genus *Thiobacillus*. pH value: 7-8

Each experimental variant represented 588 vines. The experiment was performed on whole rows in a vineyard (approximately 300 m in length). The treatment was made on four rows and the evaluation was carried out on the two middle rows, which were the same in 2023 and 2024. All vines in the row were evaluated.

The experiment also included an untreated control. The trial was conducted in the following design: row 1-8 NovaFerm® SIRIUS (5.0 l/ha), row 9-16 NovaFerm® ORION (5.0 l/ha), row 17-24 Microfertile Carbon (5.0 l/ha). The biostimulants were applied in a solo application, without tank mix with plant protection products, in a water quantity of 250 $l.ha^{-1}$.

The dates of application of biostimulants were chosen according to the growth of the vines and the risk of drought in the vineyard. The total precipitation (Fig. 1, Fig. 2) was monitored by an automatic weather station located in the vineyard (www.amet.cz). Biotimulants are used specifically to reduce impact of drought. This strategy was also chosen for application in this experiment to reduce the effects of abiotic stresses on the vines and maintain growth vigour.

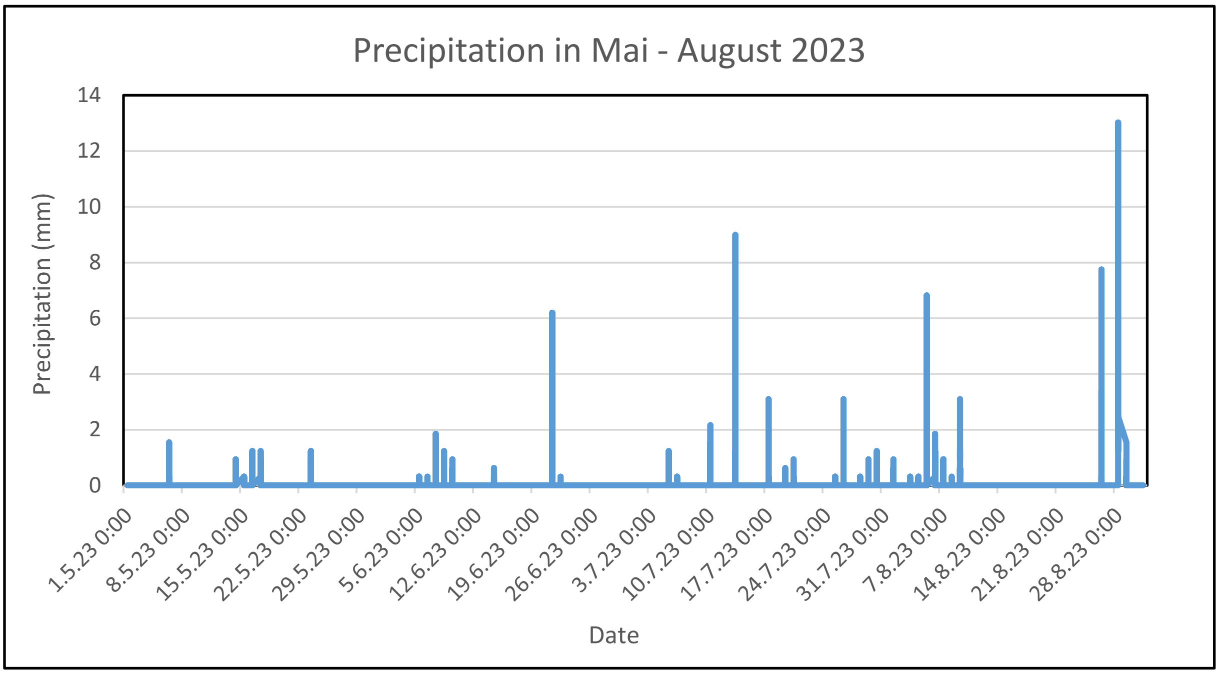

Fig. 1: Precipitation in Mai – August 2023.

In 2023, the applications of biostimulant were made on 3 July 2023 and 15 August 2023. In 2024, the applications of biostimulant were made on 5 June, 12 July and 13 August. The first application (5 June 2024) was made to stimulate vine growth, as vine growth and development was limited during April and May due to the cold weather and it was suitable to stimulate vine growth.

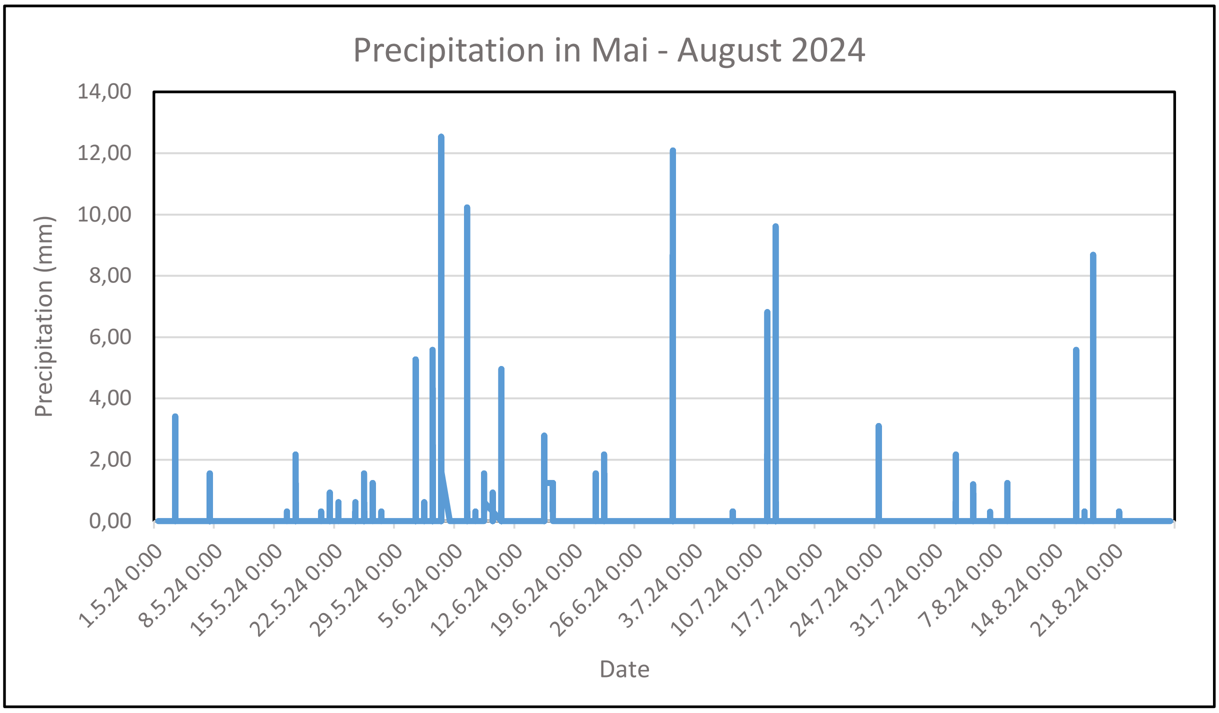

Fig. 2: Precipitation in Mai – August 2024.

The evaluations were made on 21 July, 21 August and 21 September in 2023 and on 27 July, 27 August and 26 September in 2024.

The following categories of plants were evaluated based on the visual symptoms of BN: vines without BN symptoms, vines with BN symptoms, vines with poor growth and missing vines.

The category "vines with poor growth" included vines with poor growth but without BN symptoms on leaves, annuals and bunches. The symptoms on leaves, bunches and annuals were successively evaluated during the experiment. The description of each symptom included in the evaluation is shown in Tab. 2.

Tab. 2: Bois noir symptom methodology for evaluating symptoms in the vineyard

Part of the plant	The symptoms
Leaf	• Leaves are discoloured, edges roll downward, leaf blade becomes brittle. • Leaf rolling often results in an angular shape. • Spots form on sun-exposed surfaces of the leaves, which enlarge into bands along the veins. These will gradually cover the entire leaf surface. • Colour of the spots and bands varies with the cultivar: white-fruited varieties usually have creamy-yellow spots and red-fruited cultivars have reddish spots.
Cluster	• Flowers dry out and fruit will not set on initially infected plants. • Bunches on plants that become infected later in the season will become brown and shrivelled. • Fruit will drop very easily with some varieties.
Shoot	• Canes ripen poorly and unevenly, often showing green and brown sections along the cane. • Canes with poorly ripened wood will appear rubbery, often causing a "weeping" appearance.

The results were statistically evaluated using UNISTAT Statistics Software 6.0. The variant contained 588 vines in three replicates. Each vine category was evaluated in each repetition. Kruskall-Wallis analysis enabled the determination of the effect of variant, year and variant x year. The differences between the variants were identified at a significance level of $p<0.05$ using the method of minimal significant difference. The dependencies between different categories of evaluated plants (vines without BN symptoms, vines with BN symptoms, vines with poor growth and missing vines) were determined by Pearson correlation.

Results and discussion

The occurrence of BN on the variety Chardonnay was used as a model to study the spatial distribution of symptomatic plants to provide information on the patterns of spread of such disease, how the severity of plant disease may vary in space and time, the extent to which recovered plants may resume symptoms, and the extent to which symptomatic plants may recover (Murolo et al., 2020).

The results from 2023 show that the number of symptomless plants decreased between the dates, while the number of plants with symptoms increased (Tab. 3).

Tab. 3: Assessment of BN symptoms incidence on vines in 2023. Letters indicate differences between variants in each month according to the test of minimal significant difference at the significance level p<0.05.

	Vines category	**control**	**Microfertile**	**NovaFerm Orion**	**NovaFerm Sirius**	**Kruskall-Wallis analysis**
July	Vines without symptoms	472 b	467 b	428 a	427 a	Sign.
	Vines with symptoms	62	52	62	65	n.s.
	Vines with poor growth	49	60	88	77	n.s.
	Missing vines	5 a	9 a	4 a	19 b	Sign.
August	Vines without symptoms	415	416	408	350	n.s.
	Vines with symptoms	105	93	97	137	n.s.
	Vines with poor growth	63	70	73	81	n.s.
	Missing vines	5 a	9 a	4 a	19 b	sign.
September	Vines without symptoms	302	321	349	313	n.s.
	Vines with symptoms	225 c	222 bc	177 a	192 ab	Sign.
	Vines with poor growth	56	36	32	64	n.s.
	Missing vines	5 a	9 a	4 a	19 b	Sign.

Mapping results show differences in the increase of symptomatic plants over time. The July 2023 assessment showed approximately the same number of symptomatic plants, including the untreated control.

Fig. 3 shows that the number of symptomatic plants increased dynamically for the untreated control and Microfertile Carbon. Statistically conclusive, the lowest increase in symptomatic plants was observed in the NovaFerm Orion variant. Statistical evaluation showed a conclusive difference between symptomatic plants when evaluated in September, with the lowest number of symptomatic plants in the NovaFerm Orion variant and the highest in the untreated control.

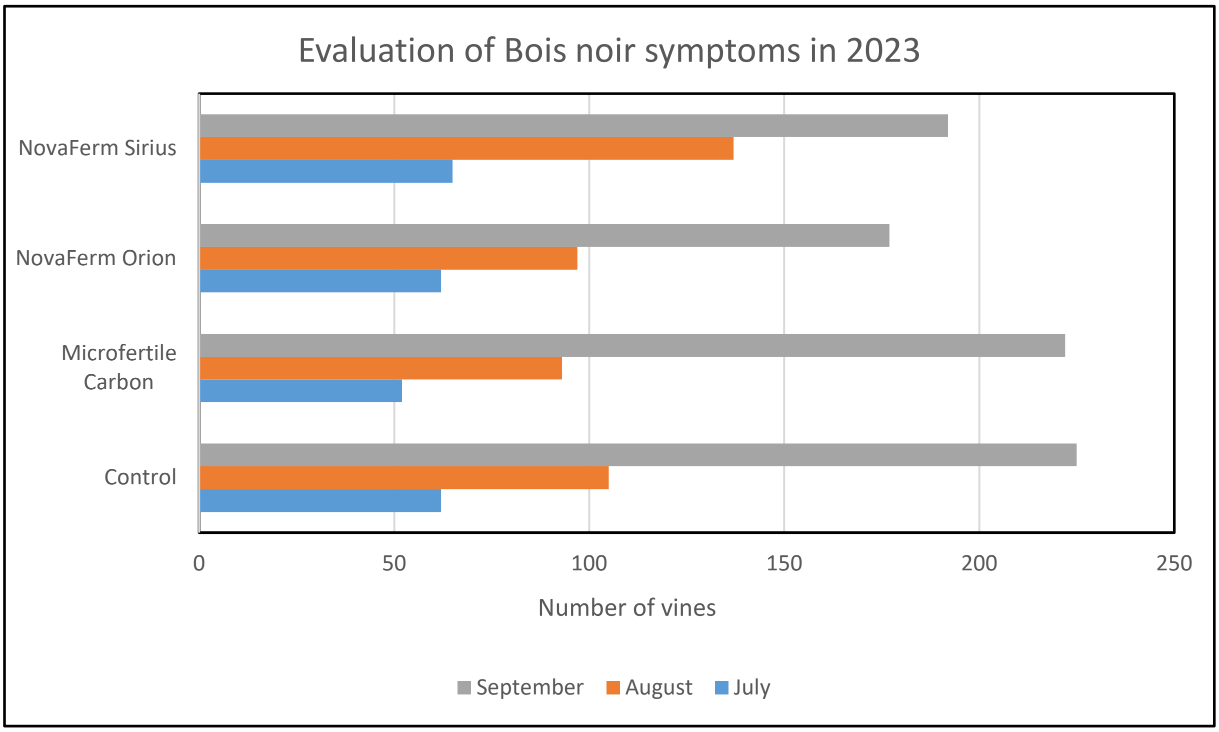

Fig. 3: Evaluation of the development of vines with Bois noir symptoms for each variant in 2023.

Also in 2024 (Tab. 4), a similar dynamic of change in the number of symptomatic plants could be observed as in 2023. However, the increase was observed in the untreated control. Similar results were confirmed in 2024, when at the September evaluation date, there were clear differences between the number of symptomatic plants, with the lowest number of symptomatic plants in the Microfertile Carbon and NovaFerm Orion variants. However, Novaferm Orion was statistically significantly different from all other variants.

Tab. 4: Assessment of symptom incidence on plants in 2024. Letters indicate differences between variants in each month according to the test of minimal significant difference at the significance level p<0.05.

	Vines category	control	Microfertile	NovaFerm Orion	NovaFerm Sirius	Kruskall-Wallis analysis
July	Vines without symptoms	398	402	388	396	n.s.
	Vines with symptoms	109	108	93	73	n.s.
	Vines with poor growth	74	69	97	99	n.s.
	Missing vines	7	9	10	20	n.s.
August	Vines without symptoms	355	381	374	341	n.s.
	Vines with symptoms	147 b	123a	125 a	149 b	Sign.
	Vines with poor growth	79	75	79	77	n.s.
	Missing vines	7	8	10	21	n.s.
September	Vines without symptoms	255 a	308 bc	329 c	270 ab	Sign.
	Vines with symptoms	286 c	237 b	215 a	252 b	Sign.
	Vines with poor growth	40	34	34	45	n.s.
	Missing vines	7	9	10	21	n.s.

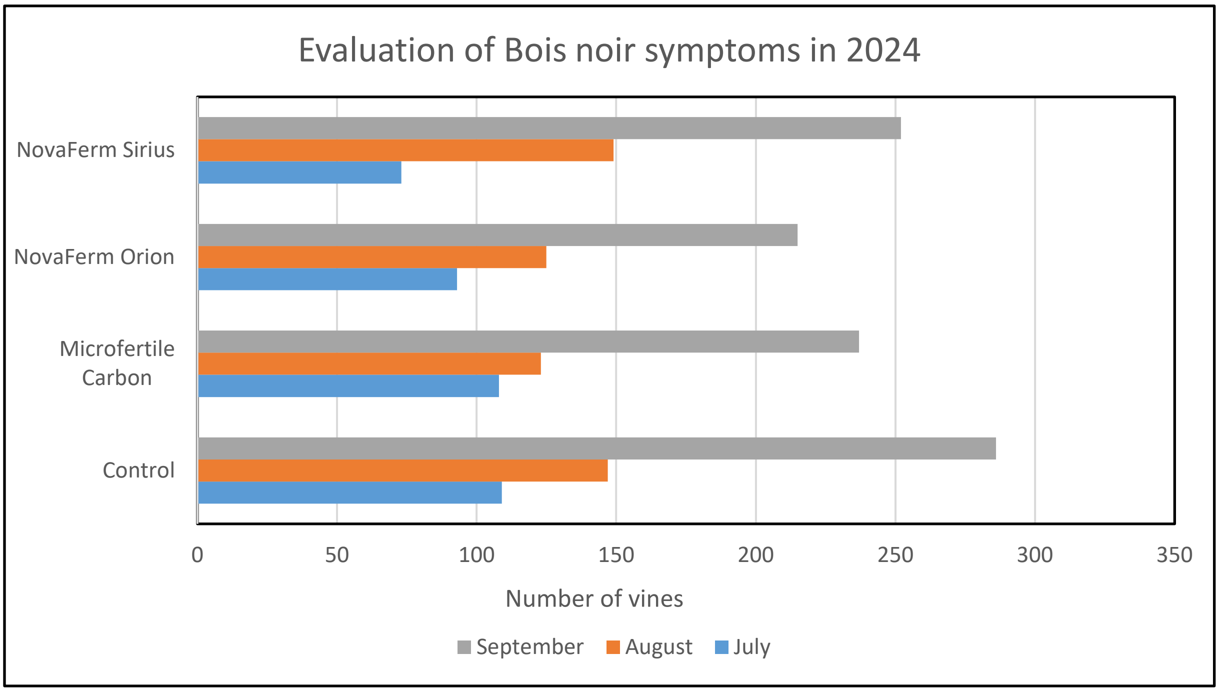

Fig. 4: Evaluation of the development of vines with Bois noir symptoms for each variant in 2024

Fig. 4 from the evaluation of the year 2024 shows that symptoms on plants appear gradually during the period July to September, with a significant increase of plants with symptoms between the August and September dates.

The number of plants with symptoms was significantly lower in the NovaFerm Orion treatment compared to the untreated control in 2023 (-21.33%) and 2024 (-24.82%). However, the comparisons with the Microfertile Carbon application are very interesting, where in 2023 there was only a minimal difference on plants with symptoms compared to the untreated control (-1.33%). However, in 2024 this difference was already significant (-17.13%). This positive effect was also evident in the comparison of plant recovery.

The lowest number of plants with symptoms in both years was found in the NovaFerm Orion variant.

The NovaFerm Orion variant also shows a significant biostimulatory effect on growth. It was found when comparing the number of poorly growing plants observed in 2023 and 2024 that the number of poorly growing plants decreases very dynamically between the evaluation dates (2023: 88-73-32, 2024: 97-79-34).

The first recorded application of elicitors was on the varieties Chardonnay and Vermentino in Sardinia, where treatment with active substances was carried out. Fosetyl-Al and a mixture of glutathione and oligosaccharides (Garau et al., 2008). Elicitor treatments can be successful in reducing the number of infected plants, reducing the severity of disease symptoms and delaying the onset of disease symptoms (Romanazzi, 2013).

Romanazzi et al. (2009) used different elicitors in 2007 and 2008, with weekly applications from June to mid-July in 2007, and then 13 applications between May and August in 2008. They carried out a relatively large number of applications.

However, in the case of microbial biostimulants in this trial, an effect was achieved in relation to reducing the development of BN with a lower number of applications. Therefore, from the point of view of the economics of viticultural production, the number of applications can be very important as it co-determines the profitability of viticultural production.

Kruskall-Wallis analysis was used to determine the effect of variant, year and variant x year on the occurrence of each plant category. In the September assessment period, a significant effect of variant and year was found in relation to plants with stolbur symptoms and plants without symptoms. For plants with stolbur symptoms, there was also a significant effect of variant x year.

The strongest correlations during all evaluation dates were between the number of plants without stolbur symptoms and the number of plants with stolbur symptoms (July: -0.7065, August: -0.9301, September: -0.8655).

Biostimulants of non-microbial origin have often been used to influence the development of stolbur symptoms or induction of recovery.

For the variety Chardonnay in the Marche region, preparations based on chitosan, fosetyl-Al, 2 formulations of glutathione plus oligosaccharines and benzothiadiazole, considered as resistance inducers, were evaluated. Application of benzothiadiazole salicyl acid reduced the number of symptomatic plants in the vineyards studied by half over the four-year trial. Resistance induction was also observed in treated plants that retained disease symptoms, as vineyard sprayed with the most effective compounds showed reduced symptom severity and more than 80% of the recovered plants showed no further symptoms in subsequent years (Romanazzi et al., 2013).

In recent years, other commercial products based on animal amino acids, seaweed extract (*Ascophyllum nodosum*) and plant amino acids, which are allowed as biostimulants in organic farming, have been evaluated as elicitors for their curative and preventive action in BN management in northern Italy. Based on the results obtained, it appeared that the reduction in the percentage of symptomatic vines was observed exclusively in vineyards treated with animal amino acids, mainly due to their curative effect (induction of recovery) (Moussa et al., 2021).

The analysis of spatial distribution can often provide insights and hypotheses about the etiology and epidemiology of diseases and the ecology of plant pathogens (Marchi et al. 2011; Murolo et al. 2014).

The evaluation was carried out by detailed mapping of individual plants in the vineyard. Fig. 5 shows the method of recording information on individual plants. It is therefore possible to make comparisons of individual plants between 2023 and 2024.

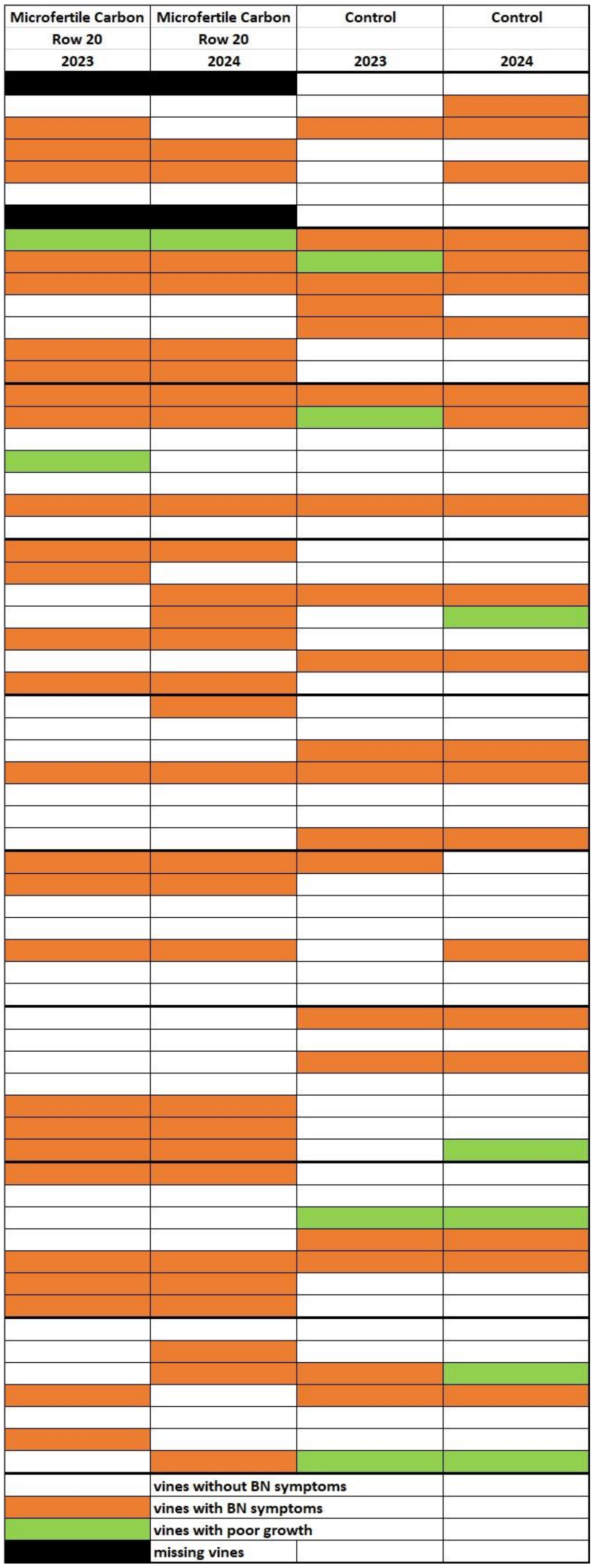

Fig. 5: Spatial mapping of plants based on categories

Tab. 5 shows the comparison of plants that recovered when comparing the years 2023 and 2024. The most significant recovery was observed for the biostimulant NovaFerm Orion. In contrast, the biostimulant Microfertile Carbon showed the least distinct change on plants with BN symptoms.

Tab. 5: Comparison of symptom change on plants year on year.

	control	**Microfertile Carbon**	**NovaFerm Orion**	**NovaFerm Sirius**
From a sick plant to a healthy one	16	16	23	16
From the healthy plant to a sick one	58	29	51	61

Although the biostimulants used influenced the number of plants with BN symptoms in each year, they showed very little effect on plant recovery. Microfertile Carbon and NovaFerm Sirius were even identical to the untreated control in the number of plants recovered. In contrast, the change from healthy to diseased plants was least dynamic with Microfertile Carbon.

It was possible to compare the same vines in both years thanks to the mapping. When comparing the same vines, most showed BN symptoms in both years.

Conclusion

The biostimulant treatment showed an effect on the number of symptomatic plants compared to the untreated control. The lowest number of plants with BN symptoms was found in NovaFerm Orion variants. NovaFerm Orion belongs to the microbial biostimulants. The NovaFerm Orion variant also shows a significant biostimulatory effect on growth, where there was a decrease in weakly growing plants during the growing season. The biostimulant Microfertile Carbon showed the most significant effect on the decrease in the number of symptomatic plants year on year. Therefore, biostimulant treatments may influence the development of symptomatic plants in the vineyard.

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